International Standard

ISO 5649

Medical laboratories — Concepts and specifications for the design, development, implementation and use of laboratory-developed tests

Laboratoires médicaux — Concepts et spécifications relatifs à la conception, au développement, à la mise en œuvre et à l'utilisation des tests développés en laboratoire

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 212, *Medical laboratories and in vitro diagnostic systems*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 140, *In vitro diagnostic medical devices,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Medical laboratory testing is carried out to an appropriate standard and all work is performed with a high level of skill and competence so as not to produce unreliable results which can lead to patient harm.

In many medical laboratories, the majority of routine clinical samples are processed and analysed using commercially available tests on automated instrumentation purchased from various manufacturers of in vitro diagnostic (IVD) medical devices. The marketing of medical devices is usually regulated by national bodies, and devices must undergo stringent assessment before they can be placed on the market and put into service.

However, there are clinical indications for which there are no commercially available IVD medical devices for the specific intended use or there is a requirement for adding additional specification/approach(es) to a commercial IVD medical device. Such tests are referred to as laboratory-developed tests (LDTs). LDTs can be defined as tests developed (or modified) and used within a laboratory to carry out testing on specimens, such as blood, body fluids and tissues, and samples derived from human specimens, such as bacterial isolates, where the results are intended to assist in clinical diagnosis or be used in making decisions concerning clinical management.

Due to technological development, advanced examinations are continuously introduced in the medical laboratory. These can include, but are not limited to liquid chromatography-tandem mass spectrometry (LC-MS/MS), time-of-flight/mass spectrometry (TOF/MS), nuclear magnetic resonance (NMR), molecular diagnostic testing (e.g. polymerase chain reaction (PCR) based and next generation sequencing (NGS)), in situ hybridization (ISH), immunohistochemistry (IHC), whole slide scanning and imaging, algorithm-based analyses and other emerging technologies. These techniques may be developed in a clinical research laboratory, transferred to the medical laboratory, and placed into routine use as diagnostic tests without going through the same standard approval processes as commercially available IVD medical devices. These tests are also considered to be LDTs.

LDTs have become more complex because of available technology and are increasingly being used to diagnose high-risk conditions such as cancer, genetic disorders, rare diseases, etc., which in turn highlights the need to ensure that the results obtained are accurate and reproducible to safeguard the health and well-being of patients. While many laboratories can perform validation studies of these tests, there is currently no international standard by which to assess the rationale for their intended use, design, development, performance, quality, and reliability.

This document is intended to be used to provide additional guidance to laboratories using LDTs. Accreditation to ISO 15189 is not a pre-requisite for laboratories to use this document.

Conceptually, the lifecycle of an LDT involves sequential phases that extend from the feasibility assessment to the final retirement of the examination procedure. The main phases of a typical LDT lifecycle described in this document, therefore, include the feasibility assessment, the design and development phase, the preliminary/pilot testing followed by the performance evaluation phase, including validation and the verification phases, the monitoring and review activities during LDT use, and the final retirement of the LDT. The illustration shown in Figure 1 below demonstrates these different phases and indicates which clauses of this document cover the corresponding lifecycle phases for an LDT. The arrows back to previous phases within Figure 1 indicate an iterative, dynamic process which can include look-backs, rework or revalidation for improvement of the LDT.

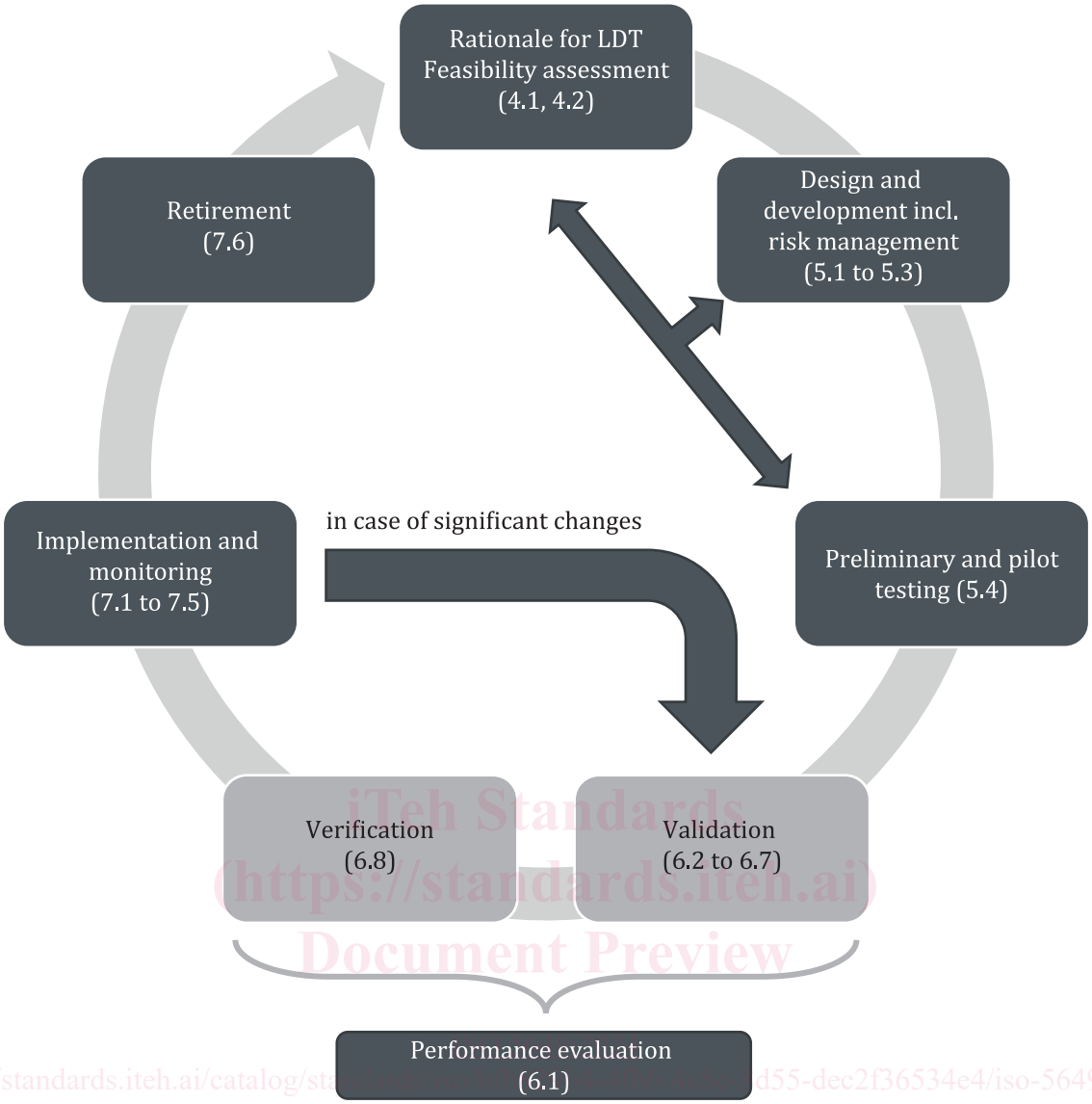

Figure 1 — Possible lifecycle phases of an LDT

The rationale for the use of an LDT and the feasibility assessment consider the demand for an LDT and determine whether analytical and clinical performance of the new LDT can meet requirements for adequate measurement procedure results (refer to 4.1 and 4.2 of this document).

Design and development include the planning and definition of formal specifications for LDT performance including iterative improvement of all LDT components according to the intended use of the LDT. This can include redesign and reassessment of feasibility and the formal specifications of the LDT as a dynamic process covering all aspects of the LDT development (refer to 5.1 to 5.3 of this document).

Preliminary testing precedes the performance evaluation phase and determines the technical aspects of the LDT by demonstrating that the LDT meets the design and development requirements (refer to 5.4 of this document).

Performance evaluation includes the collection, analysis and assessment of performance data typically generated from validation and verification studies, but also includes activities of risk management and supports the demonstration of the conformity of the LDT to applicable principles of safety and performance.

Validation is a defined process to confirm and control that the finally designed and developed LDT is suitable for its intended use and fulfils all analytical and clinical performance claims (refer to 6.2 to 6.7 of this document).

LDT specifications are verified, where relevant aspects of the LDT procedure deviate between the phase of validation and routine use of the LDT (refer to 6.8 of this document for verification).

LDTs are continuously monitored and periodically reviewed to ensure conformity with the original performance specifications. Significant changes of the LDT require a restart of the processes affected by the modification including revalidation (refer to 7.1 to 7.5 of this document for implementation and monitoring).

LDTs that need replacement shall be retired (refer to 7.6 of this document for retirement).

An example for how this lifecycle can be applied to a workflow is presented in Annex A of this document.

Medical laboratories — Concepts and specifications for the design, development, implementation and use of laboratory-developed tests

1 Scope

This document establishes requirements for assuring quality, safety, performance and documentation of laboratory-developed tests (LDTs) as per their intended use for the diagnosis, prognosis, monitoring, prevention or treatment of medical conditions.

It outlines the general principles and assessment criteria by which an LDT shall be designed, developed, characterized, manufactured, validated (analytically and clinically) and monitored for internal use by medical laboratories.

The scope includes regulatory authority approved IVD medical devices that are used in a manner differing from approved labelling or instructions for use for that device (e.g. use of a sample type not included in the intended use, use of instruments or reagents not included in the labelling).

While this document follows a current best practice and state-of-the art approach, it does not provide specific details on how to achieve these requirements within specific disciplines of the medical laboratory nor specific technology platforms.

This document does not specify requirements for examination procedures developed by research or academic laboratories developing and using testing systems for non-IVD purposes. However, the concepts presented in this document can also be useful for these laboratories.

This document does not apply to the design, development and industrial production of commercially used IVD medical devices.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
analyte
component represented in the name of a measurable quantity

EXAMPLE In “mass of protein in 24-hour urine”, “protein” is the analyte. In “amount of substance of glucose in plasma”, “glucose” is the analyte. In both cases, the long phrase represents the *measurand* (3.28)

[SOURCE: ISO 17511:2020, 3.1]

3.2
analytical performance
analytical performance of an LDT
ability of a *laboratory-developed test (LDT)* (3.25) to detect or measure a particular *analyte* (3.1)

Note 1 to entry: *Clinical evidence* (3.7) of an LDT is composed of three elements: *scientific validity* (3.51), analytical performance and *clinical performance* (3.8).

[SOURCE: IMDRF/GRRP WG/N47:2018, 3.2[20], modified — "IVD medical device" was replaced by "LDT".]

3.3
analytical sensitivity
sensitivity of a measurement procedure
quotient of the change in a measurement indication and the corresponding change in a value of a quantity being measured

Note 1 to entry: The analytical sensitivity can depend on the value of the quantity being measured.

Note 2 to entry: The change considered in the value of the quantity being measured shall be large compared with the resolution.

Note 3 to entry: The analytical sensitivity of a measuring system is the slope of the calibration curve.

Note 4 to entry: Analytical sensitivity should not be used to mean *detection limit* (3.13) or *quantitation limit* (3.43) and should not be confused with *diagnostic sensitivity* (3.15).

[SOURCE: ISO 18113-1:2022, 3.2.4]

3.4
analytical specificity
selectivity of a measurement procedure
capability of a measuring system, using a specified *measurement procedure* (3.31), to provide measurement results for one or more *measurands* (3.28) which do not depend on each other nor on any other quantity in the system undergoing measurement

EXAMPLE Capability of a measuring system to measure the concentration of creatinine in blood plasma by the alkaline picrate procedure without interference from the glucose, urate, ketone, or protein concentrations.

Note 1 to entry: Lack of analytical specificity is called analytical interference.

Note 2 to entry: Lack of analytical specificity in immunochemistry measurement procedures can be due to *cross-reactivity* (3.11).

Note 3 to entry: Specificity of a measurement procedure should not be confused with *diagnostic specificity* (3.16).

Note 4 to entry: ISO/IEC Guide 99:2007 uses the term selectivity for this concept instead of specificity.

[SOURCE: ISO 18113-1:2022, 3.2.5]

3.5
bias
measurement bias
estimate of a systematic measurement error

Note 1 to entry: See also ISO/IEC Guide 99:2007, 2.17, systematic measurement error.

Note 2 to entry: This definition applies to quantitative measurements only.

[SOURCE: ISO 15189:2022, 3.1, modified — a new Note 1 to entry was added and former Note 1 to entry is Note 2 to entry.]

3.6
biological reference interval
reference interval
specified interval of the distribution of values taken from a biological reference population

Note 1 to entry: A biological reference interval is commonly defined as the central 95 % interval. Another size or an asymmetrical location of the biological reference interval can be more appropriate in particular cases.

Note 2 to entry: A biological reference interval can depend upon the type of primary sample and the *examination* (3.17) procedure used.

Note 3 to entry: In some cases, only one biological reference limit is important, usually an upper limit, "x", so that the corresponding biological reference interval would be less than or equal to "x".

Note 4 to entry: Terms such as 'normal range', 'normal values', and 'clinical range' are ambiguous and therefore discouraged.

[SOURCE: ISO 15189:2022, 3.2]

3.7
clinical evidence
information that supports the *clinical utility* (3.9) of a *laboratory-developed test (LDT)* (3.25) for its *intended use* (3.22), including *scientific validity* (3.51), *analytical performance* (3.2), and *clinical performance* (3.8)

3.8
clinical performance
clinical performance of a laboratory-developed test (LDT)
ability of a *laboratory-developed test (LDT)* (3.25) to yield results that are correlated with a particular clinical condition/physiological state in accordance with target population and intended user

Note 1 to entry: Clinical performance can include *diagnostic sensitivity* (3.15) and *diagnostic specificity* (3.16) based on the known clinical or physiological state of the individual, and *negative predictive values* (3.37) and *positive predictive values* (3.42) based on the prevalence of the disease.

Note 2 to entry: The clinical performance of an LDT is also referred to as the ability of a test to discriminate between the target condition and health.

[SOURCE: IMDRF/GRRP WG/N47:2018, 3.11[20], modified — "IVD Medical Device" was replaced by "LDT" and Note 2 was replaced.]

3.9
clinical utility
usefulness of the results obtained from testing and the value of the information to either the individual being tested or the broader population, or both

Note 1 to entry: Aside from *scientific validity* (3.51), *analytical performance* (3.2), and *clinical performance* (3.8), a laboratory is not required to demonstrate any other elements of the clinical utility of a *laboratory-developed test (LDT)* (3.25).

Note 2 to entry: Adapted from GHTF/SG5/N6:2012, 4.7[23].

3.10
competence
demonstrated ability to apply knowledge and skills to achieve intended results

[SOURCE: ISO 15189:2022, 3.5]

3.11
cross-reactivity
degree to which a substance other than the *analyte* (3.1) intended to be measured affects an *examination* (3.17) procedure

EXAMPLE Antibody binding to metabolites of the analyte, structurally similar drugs, etc.

Note 1 to entry: *Analytical specificity* (3.4) is a related concept.

Note 2 to entry: Cross-reactivity of metabolites can be a desirable attribute of certain examination procedures, such as for screening for the presence of illegal drugs.

Note 3 to entry: It is important to calculate cross-reactivity on the basis of moles per litre. For guidelines in calculating cross-reactivity, see Reference [54].

[SOURCE: ISO 18113-1:2022, 3.2.14, modified — "binds to a reagent in a competitive binding immunochemical measurement procedure" was replaced by "intended to be measured affects an examination procedure".]

3.12
design and manufacture
activities that may include specification development, production, assembly, processing, sterilization, installation of a *laboratory-developed test (LDT)* (3.25) or putting a collection of devices, and possibly other products, together for a medical purpose as specified by the laboratory

[SOURCE: IMDRF/GRRP WG/N47:2018, 3.25[20] NOTE 3, modified — "medical device" was replaced by "LDT".]

3.13
detection limit
limit of detection
measured quantity value, obtained by a given *measurement procedure* (3.31), for which the probability of falsely claiming the absence of a component in a material is β, given a probability α of falsely claiming its presence

Note 1 to entry: IUPAC recommends default values for α and β equal to 0,05.

Note 2 to entry: The abbreviation LoD is sometimes used.

Note 3 to entry: The term "sensitivity" is discouraged for 'detection limit'.

[SOURCE: JCGM 200:2012, 4.18[19]]

3.14
diagnostic accuracy
extent of agreement between the information from the test under evaluation and applicable performance attributes as measured by a reference method

Note 1 to entry: Diagnostic accuracy can be expressed in different ways, including sensitivity-specificity pairs, likelihood ratio pairs, and the area under a receiver operating characteristic curve.

Note 2 to entry: Diagnostic accuracy shall be interpreted in context with the condition of interest and the combination of specific criteria and methods used.

Note 3 to entry: Diagnostic accuracy is not the same as *measurement accuracy* (3.29), which is the closeness of a single result of a measurement and a true value.

[SOURCE: CLSI Harmonized Terminology Database,[49] Project: EP12, M55, modified — "diagnostic accuracy criteria" has been replaced by "applicable performance attributes as measured by a reference method".]

3.15
diagnostic sensitivity
ability of an *examination* (3.17) procedure to have positive results associated with a particular disease or condition

Note 1 to entry: Also defined as percent positivity in samples where the target marker is known to be present. For information regarding description of the diagnostic *performance characteristics* (3.39) of a *laboratory-developed test (LDT)* (3.25), see Reference [55].

Note 2 to entry: Diagnostic sensitivity is expressed as a percentage (number fraction multiplied by 100), calculated as 100 × the number of true positive values (TP) divided by the sum of the number of true positive values (TP) plus the number of false negative values (FN), or 100 × TP / (TP + FN). This calculation is based on a study design where only one sample is taken from each subject.